ESRF	**Experiment title:** Low Resolution Phasing of Datasets of Human LDL Particles by MAD and MASC	**Experiment number**: LS 1438
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Report:

LDL particles play a major role in the development of coronary heart disease. A certain subclass of LDL particles, (small, dense LDL) has been identified as a major determinant of the severity and progression of atherosclerosis in humans [1]. Solving the structure of LDL, respectively its protein component apoB, is of basic relevance as it represents a novel kind of macromolecular assembly, for which no high resolution structures are known. In addition, the analysis of the three-dimensional structure of apoB is an important approach to explain the mechanisms involved in the development of atherosclerosis on a molecular basis. Of special interest would be to compare the structure of apoB in LDL particles from LDL subfractions of different atherogenic potential [1].

In previous experiments we developed the methodology [2] to collect, index, integrate, scale and merge complete datasets of LDL-crystals with all inner reflections resolved. *The method is of interest for work on other large macromolecular complexes*. Currently the main challenge, besides improvement of resolution, is to assign phases to these datasets. A mathematical approach that is currently followed, is to use direct methods [3].

In the present experiment, it was planed to use two experimental methods MAD and MASC [4] get experimental phases. The rationale for doing MAD and MASC is that they give complementary information on the structure of LDL particles at low resolution. MASC, if successful, provides the shape of the particles which is currently not well defined in the

solution found by direct methods. For the *MASC experiment* native LDL crystals were soaked with molar solutions of 4 different promising substances. Unfortunately none of the crystals diffracted.[i] Further experiments will focus on improved methods of soaking and the test of other compounds.

In the *MAD experiment* the presence of the covalently linked gold label could be confirmed by X-ray fluorescence spectra. In the available time MAD datasets of 2 LDL-crystals could be collected at 4 wavelength under cryogenic conditions. For each dataset 2 passes were necessary (10s, $\Delta\varphi=1°$ and 0.5s $\Delta\varphi=2°$, $\varphi=0\text{-}180°$) to collect the inner reflections with a few overloaded pixels only. Measurement of the innermost reflections was possible by using a heliumpath and a small beamstop in front of the imageplate as previously described [2].
Evaluation of the datasets is currently in progress. One binding site has been identified in the anomalous difference Patterson maps and refined by SHARP. The calculated electron density maps show connected areas of density compatible with the model of LDL as resulting from small angle X-ray scattering [5]. The results however, suggest that data quality should be further improved by increasing the signal to noise ratio preferably by data collection with higher redundancy. In addition a third pass with an attenuated beam could be helpful to have no overloaded reflections at all, as even a few overloaded reflections can have a strong impact on the resulting electron density at low resolution [6].
The next experiments will focus on further improving the data quality of the MAD datasets, the identification of suitable substances for MASC experiments and the improvement of resolution by various strategies.

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[i] It has to be noted that diffraction tests of LDL crystals can only be made at synchrotron sources, due to the low scattering power (large unit cell, low protein content) of the crystals.